AMBER 9.0 Drug/DNA Complex Tutorial

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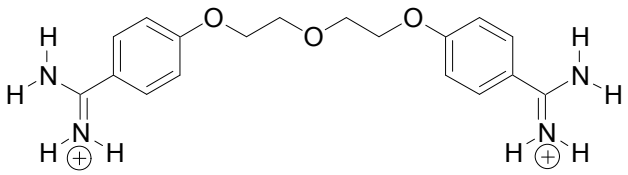
Synopsis. Today you will learn how to use one of the most versatile molecular dynamics modeling packages, AMBER 9. [1] The AMBER suite of programs was developed by the late Peter A Kollman and colleagues at the University of California San Francisco (UCSF) and is now maintained at Scripps Institute. See the Amber webpage at http://amber.scripps.edu for more information. The Amber package was designed with the ability to address a wide variety of biomolecules including proteins and nucleic acids as well as small molecule drugs.

Research Problem. We will work on the complex of a DNA minor groove binding drug (γ-oxa-pentamidine). We will take the x-ray crystal structure (166D.PDB) [2] of the complex, and study its behavior in water using molecular dynamics. Make a new project directory using the "mkdir" command. Copy 166D.pdb and pet.pdb to this directory.

Preparation of the drug. Open your pet.pdb file in MOE.

Open MOE. Go to File > Open > pet.pdb (Click on Auto Connect and Center in the dialog).



Open the Builder by Clicking on the Builder … button on the right-hand side of the MOE window. Change the formal charge of one of the nitrogens of each aminidinium group to +1 (i.e. one nitrogen on each side of the molecule). Use the Element: tools in *Molecule Builder*.

Edit > Add Hydrogens

IMPORTANT: *Each atom of the drug molecule must have a unique name*! MOE does not do this automatically! Click on Labels > Name to display the atom names. Select each Hydrogen atom one-by-one and give it a unique name using the atoms dialog. Select the atom with your mouse (turns pink) then Click on Atoms (right-hand side menu) > *Atom Manager* Click on Selection only. Highlight the hydrogen atom in the Atom Manager and change the name from H to H# (where # is the next number in the sequence; examine to see if the other hydrogen atoms are numbered otherwise start numbering from 1.). When you are finished, replace the existing file with the new file.

File > Save As > File Format: PDB : pet.pdb

The table below shows the AMBER names for nucleic acid residues. Those names with a D are for DNA residues and those with an R are for RNA residues. A terminal 5′ residue would be indicated by e.g. DA5 and a 3′ residue would be e.g. DT3. However, Leap will recognize the normal nucleic acid names given and assume them to be DNA. Leap is able to differentiate 3′ from 5′ residues.

Group or Residue	*Residue Name, Alias*
Adenine	DA, RA
Thymine	DT
Uracil	RU
Cytosine	DC, RC
Guanine	DG, RG

Open 166D.pdb in nedit and open pet.pdb in nedit. Replace the old "PET" residue coordinate records with the new PET coordinates. Save the new file as "dna_pet.pdb".

Making a special topology "prep" input file to describe the drug. AMBER has a residue topology database to describe all of your nucleic acid residues as well as amino acid residues if you happen to be working with a protein. However, AMBER does not have a special topology database to describe your drug. Fortunately, AMBER has a special program called "**antechamber**", which is designed to build a topology prep file for your drug. [3] In combination with DivCon (a semi-empirical QM program), antechamber will compute partial atomic charges for your drug. We will use AM1-BCC charges[4], as these charges scale best with the charge set we will be using on our DNA. By default, antechamber will write a prep file with the *general amber force field* (gaff) atom types. To write a prep file with regular Amber atom types, use the **–at** *amber* flag. However, always use the *gaff* force field for your small molecules. Issue the following command from within your working directory.

```
antechamber -nc 2 -rn PET -i pet.pdb -fi pdb -o pet_bcc.prep -fo prepi -c bcc
```

What the command line flags mean:

-nc is the net molecular charge (very important!)
-rn is the residue name (default is MOL) Use a name here that you know is used in your pdb file!
-i is the input file
-fi is the input file type (e.g. *pdb, mol2*; see AMBER manual for more info)
-o is the output file name
-fo is the output file type (e.g. *prepi* for AMBER prep file)
-c is the charge type used (*bcc* will compute AM1-BCC charges; *rc* will read in charges from the input file; see AMBER manual for more info)

Contents of the prep file

```
    0    0    2

This is a remark line
molecule.res
PET    XYZ  0
CORRECT     OMIT DU   BEG
  0.0000
   1  DUMM  DU    M    0  -1  -2     0.000      .0        .0      .00000
   2  DUMM  DU    M    1   0  -1     1.449      .0        .0      .00000
   3  DUMM  DU    M    2   1   0     1.522   111.1        .0      .00000
   4  N1    nh    M    3   2   1     1.540   111.208   180.000    -0.456
   5  H4    hn    E    4   3   2     1.010    59.922  -148.384     0.334
... etc
   #   name type  ts connectivity    bnd len   angle    dihedral     charge

LOOP (Describe ring (i.e. cyclic) structures)
   C1   C6
  C13   C8

IMPROPER (Improper dihedrals)
   C4   N1   C7   N2
   C7   C5   C4   C3
   C4   C6   C5   H5
   C5   C1   C6   H6
   C4   C2   C3   H7
   C3   C1   C2   H8
   C6   C2   C1   O1
   C9  C13   C8   O2
   C8  C10   C9  H17
   C9  C11  C10  H18
  C14  C10  C11  C12
  C11   N3  C14   N4
  C11  C13  C12  H20
   C8  C12  C13  H19

DONE
STOP
```

- atom number for atom I (can be any atom)
name – atom name for atom I
type – atom type for atom I
ts – Tree Structure – Describes the geometry of the structure and how it links to the rest of the "larger" structure if this prep file describes a residue. There are 5 topological types: Main, Side, Branch, (3, 4, 5, & 6), and End or **M**, **S**, **B**, 3 etc., **E**.

Main atoms describe the "path" through the residue connecting it to the next residue.

E is for the *End* atom of a chain. The E atoms can only have one connection to another heavy atom. **S** (side) must have connections to two other atoms. **B** (branch) must have connections to three other heavy atoms. A 3 atom has a total of four connections to heavy atoms (a quaternary atom). The 4, 5, and 6 are used to describe higher order bonding (e.g. metal complexes).

LOOP closing section describes cyclic systems. Loop connections are not counted when assigning M, S, B, etc.

DUMMY ATOMS – Three are required. These are used to define the space axes for the residue. They must be given the topological description of "M", and must be of the "Dummy" atom type, DU.

connectivity – Here is what the 3 numbers mean in reference to atom I:

NA(I) 1st number – The atom # to which atom I is connected.
NB(I) 2nd number – The atom # to which atom I makes an angle along with NA(I)
NC(I) 3rd number – The atom # to which atom I makes a dihedral with NA(I) & NB(I).

bnd len – Equilibrium bond length between atom I and NA(I).

angle – The bond angle between NB(I), NA(I) and I.

dihedral – The dihedral angle between NC(I), NB(I), NA(I), and I

charge – The partial atomic charge on I.

LOOP – Describes a loop closing bond for each cyclic ring in the molecule.

IMPROPER – Tells amber which 'improper' torsion angles are to be used for the calculation. In united atom models, improper torsions are used to keep asymmetric centers from racemizing. In addition improper torsions are used to enforce planarity in cyclic ring systems in both united and all atom models. The improper torsions are to be defined in such a way that the proper torsions are not duplicated.

Use **parmchk** to check the parameters.

```
parmchk -i pet_bcc.prep -f prepi -o pam.frcmod
```

Check the *pam.frcmod* file contents (use **more** pam.frcmod). You will notice that there are a few parameters that needed estimates based on existing parameters. The output has no warnings about unknown atoms or parameters. Therefore, we may proceed.

Contents of the pam.frcmod file:

```
remark goes here
MASS

BOND

ANGLE
nh-ce-nh   72.900     117.500   same as nh-c2-nh
nh-ce-ca   66.206     117.715   Calculated with empirical approach

DIHE
nh-ce-nh-hn   1    0.675       180.000          2.000      same as X -c2-nh-X
nh-ce-ca-ca   1    2.550       180.000          2.000      same as X -c2-ca-X
hn-nh-ce-ca   1    0.675       180.000          2.000      same as X -c2-nh-X
```

```
IMPROPER
ca-nh-ce-nh         1.1          180.0         2.0          Using default value
ca-ca-ca-ce         1.1          180.0         2.0          Using default value
ca-ca-ca-ha         1.1          180.0         2.0          General improper torsional
angle (2 general atom types)
ca-ca-ca-os         1.1          180.0         2.0          Using default value

NONBON
```

xLeap and tLeap – These programs perform the same function with the difference being that xLeap opens in an x-window interface and tLeap operates from a terminal prompt (no GUI). The principal function of these programs is to prepare the AMBER coordinate (inpcrd) and topology (prmtop) files.

```
xleap -s -f leaprc.ff99
```

Adjust for use of the SPCE/E water model.

```
HOH=SPC
WAT=SPC
loadamberparams frcmod.spce
```

The **leaprc.ff99** file loads the parameters for the AMBER99 force field. [5, 6] The **leaprc.gaff** file loads the parameter set for the *general amber force field,* which is used to describe your small molecule.

In the leap window or prompt type the following commands one at a time:

```
source leaprc.gaff

loadAmberParams pam.frcmod

loadAmberPrep pet_bcc.prep

nap = loadPdb dna_pam.pdb

check nap
```

The check reveals a warning for the charge of the unit as being nonzero. We will add counterions later to neutralize.

```
edit nap
```

The following mouse buttons perform as indicated:

LEFT -	Selection
MIDDLE -	Rotate
RIGHT -	Translate
MIDDLE + RIGHT -	Zoom In and Out

The structure should be fine at this point. You should notice that Leap has added hydrogen atoms to the DNA structure. To exit the unit editor use Unit > close from the menu.

Solvate the structure in SPC/E water [7] using a cubic periodic box. Use the following command;

```
solvateBox nap SPCBOX 9.0
```

You have told Leap to solvate the unit in a cubic box using a spacing distance of 9.0 angstroms around the molecule. Ideally, you should set the spacing at no less than 8.5 Å (~ 3 water layers) to avoid periodicity artifacts. [8] For particle-mesh ewald electrostatics, [9, 10] our box side length must be > 2 X NB cutoff. We will use a 9.0 Å cutoff; therefore, our box side must be > 18 Å. Our box side length will be (2 X 8) + DNA dimension, which should be greater than 18. Now, lets add counterions to neutralize the charge. We had a total charge of –20.000; therefore, we need 20 sodium ions to neutralize.

```
addIons nap Na+ 0
```

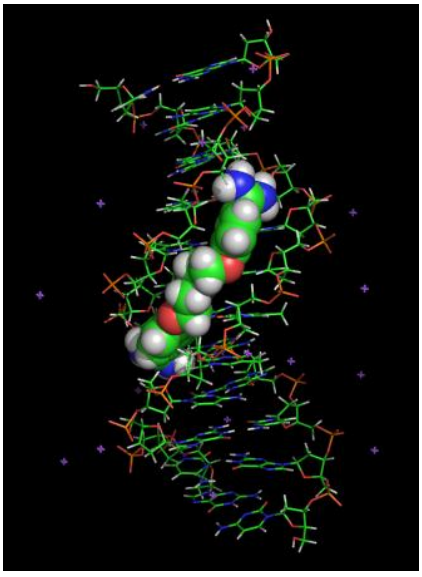
The command we just issued (addIons) added Na+ ions until the total net formal charge = 0. This should add 20 Na+ ions. Now we can save the topology and coordinate files!

```
saveAmberParm nap pet.top pet.crd
```

As an additional step, you may want to save a PDB file of the model you just created. To do this, just use the savePdb command.

```
savePdb nap pet_na.pdb
```

You're done with file preparation (hooray!). Type "**Quit**" to exit xLeap!
Use Rasmol or Pymol to view your model. In the *winterm*, type "**rasmol** pet_na.pdb". Our model before dynamics …



Molecular Dynamics

We'll do these computations in 4 steps. The first three steps are preparatory and the last step is what is known as the "production run".

Step 1. *Energy Minimization of the System*. We perform a steepest descents energy minimization to relieve bad steric interactions that would otherwise cause problems with or dynamics runs.

Using the Sander program. The SANDER program is the number crunching juggernaut of the AMBER software package. SANDER will perform energy minimization, dynamics and NMR refinement calculations. You must specify an input file to tell SANDER what computations you want to perform and how you would like to perform those computations. Study the input file for energy minimization below.

The contents of the min1.in file.

```
Initial restrained minimization on DNA & DRG, 10 cut
 &cntrl
  imin = 1,
  maxcyc = 1000,
  ncyc = 250,
  ntb = 1,
  ntr = 1,
  cut = 10
 /
Hold the DNA & DRG fixed
500.0
RES   1   25
END
END
```

What it all means in a nutshell. For more in-depth info please consult the AMBER User's Manual. The settings important for minimization are highlighted and explained below.

&cntrl and / – Most if not all of your instructions must appear in the "control" block (hence &cntrl).

cut = nonbonded cutoff in angstroms.
ntr = Flag used to perform position restraints (1 = on, 0 = off)
imin = Flag to run energy minimization (if = 1 then perform minimization; if = 0 then perform molecular dynamics).
macyc = maximum # of cycles
ncyc = After ncyc cycles the minimization method will switch from steepest descents to conjugate gradient.
ntmin = Flag for minimization method. (if = 0 then perform full conjugate gradient min with the first 10 cycles being steepest descent and every nonbonded pairlist update; if = 1 for ncyc cycles

steepest descent is used then conjugate gradient is switched on [default]; if = 2 then only use steepest descent)
dx0 = The initial step length
dxm = The maximum step allowed
drms = gradient convergence criterion 1.0E-4 kcal/mol•Å is the default

Hold the DNA and the DRG fixed
500.0 (This is the force in kcal/mol used to restrain the atom positions.)
RES 1 25 (Tells AMBER to apply this force to residue #'s 1 to 25).

Command line in general (Example only):

```
sander -O -i min.in -o min.out -p prmtop -c inpcrd -r restrt [-ref refc -x
mdcrd -v mdvel -e mden -inf mdinfo]
```

Issue the following command to run your energy minimization.

```
nohup sander -O -i min1.in -o min1.out -p pet.top -c pet.crd -r pet_min1.rst
-ref pet.crd &
```

Monitor the progress using "**tail –20** min1.out" command.
Step 2. Minimization of whole system, no restraints.

Contents of min2.in file

```
System minimization on DNA & DRG, 10 cut
 &cntrl
  imin = 1,
  maxcyc = 1500,
  ncyc = 500,
  ntb = 1,
  ntr = 0,
  cut = 10
 /
END
```

```
nohup sander -O -i min2.in -o min2.out -p pet.top -c pet_min1.rst -r
pet_min2.rst &
```

Step 3. *Position-Restrained Dynamics*. This initial dynamics run is performed to relax the positions of the solvent molecules. In this dynamics run, we will keep the macromolecule atom positions restrained (not fixed, however). In a position-restrained run, we apply a force to the specified atoms to minimize their movement during the dynamics. The solvent we are using in our system, water, has a relaxation time of 10 ps; therefore we need to perform at least > 10 ps of position restrained dynamics to relax the water in our periodic box.

Contents of md1.in

```
position restrained dynamics, model 1, 10.0 cut
 &cntrl
  imin = 0,
```

```
  irest = 0,
  ntx = 1,
  ntb = 1,
  cut = 10,
  ntr = 1,
  ntc = 2,
  ntf = 2,
  tempi = 0.0,
  temp0 = 300.0,
  ntt = 3,
  gamma_ln = 1.0,
  nstlim = 12500, dt = 0.002,
  ntpr = 100, ntwx = 500, ntwr = 1000
/
Hold the drug and DNA fixed
10.0
RES  1  25
END
END
```

ntb = 1 Constant volume dynamics.
imin = 0 Switch to indicate that we are running a dynamics.
nstlim = # of steps limit.
dt = 0.002 time step in ps (2 fs)
temp0 = 300 reference temp (in degrees K) at which system is to be kept.
tempi = 0 initial temperature (in degrees K)
ig = ### seed for random number generator
heat = 0 multiplier for velocities (default = 0.0).
ntt = 3 temperature scaling switch (1 = Langevin Dynamics)
gamma_ln = 1.0 collision frequency in ps^{-1} when ntt = 3 (see Amber 8 manual).
tautp = 1.0 Time constant for the heat bath (default = 1.0) smaller constant gives tighter coupling.
vlimit = 20.0 used to avoid occasional instability in dynamics runs (velocity limit; 20.0 is the default). If any velocity component is > vlimit, then the component will be reduced to vlimit.
comp = 44.6 unit of compressibility for the solvent (H_2O)
ntc = 2 Flag for the Shake algorithm (1 – No Shake is performed; 2 – bonds to hydrogen are constrained; 3 – all bonds are constrained).
tol = #.##### relative geometric tolerance for coordinate resetting in shake.

The additional blocks that follow are the nmr restraint instructions. We will gradually warm the system from 100 K to 300 K in the first picosecond; followed by maintaining the temperature at 300 K.
You will note that we used a smaller restraint force (10.0 kcal/mol). For dynamics, one only needs to use 5 to 10 kcal/mol restraint force when **ntr = 1** (uses a harmonic potential to restrain coordinates to a reference frame; hence, the need to include reference coordinates with the –ref flag.). Larger restraint forces lead to instability in the shake algorithm with a 2 fs time step. Larger restraint force constants lead to higher frequency vibrations, which in turn lead to the instability. Excess motion away from the reference coordinates is not possible due to the steepness of the harmonic potential. Therefore, large restraint force constants are not necessary.

```
nohup sander -O -i md1.in -o md1.out -p pet.top -c pet_min2.rst -r
pet_md1.rst -x pet_md1.mdcrd -ref pet_min2.rst -inf md1.info &
```

Monitor the progress using "**tail –40** md1.out". Our computation took about 1.8 hours to complete on a 2.7 GHz linux workstation.

Step 4. *The Production Run*. This is where we do the actual molecular dynamics run. You will do a 100 ps run.

Contents of md2.in

```
dynamics w/PME, DNA-Drug, 10.0 cut, 50 ps
 &cntrl
  imin = 0, irest = 1, ntx = 7,
  ntb = 2, pres0 = 1.0, ntp = 1,
  taup = 2,
  cut = 10, ntr = 0,
  ntc = 2, ntf = 2,
  tempi = 300.0, temp0 = 300.0,
  ntt = 3, gamma_ln = 1.0,
  nstlim = 50000, dt = 0.002,
  ntpr = 100, ntwx = 500, ntwr = 1000
/
END
```

ntp = 1 md with isotropic position scaling.
ntb = 2 constant pressure
pres0 = 1 reference pressure in bar
taup = 2.0 pressure relaxation time in ps

Issue the following command to run your dynamics simulation.

```
nohup sander -O -i md2.in -o md2.out -p pet.top -c pet_md1.rst -r pet_md2.rst
-x pet_md2.mdcrd -ref pet_md1.rst -inf md2.info &
```

Monitor the progress using "**tail –40** md2.out". Our 100 ps run took approximately 11 hours to run on a 2.7 GHz linux workstation.

Analysis

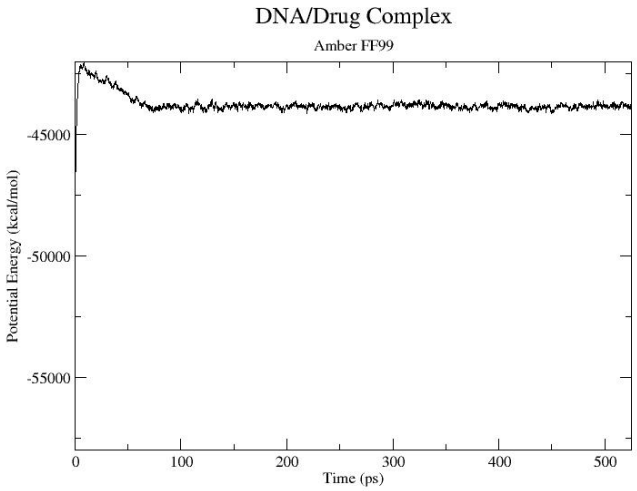
Note: The sample data presented here will be different from your data if you started with crystal structure. We used the result from an Autodock docking as our starting complex based on the same crystal structure.

Copy **process_mdout.perl** to your working project directory. Use the "which" command to determine the location of your perl interpreter (type "**which** perl"). On the SGI, the location (after #!) should be /usr/sbin/perl. Check to make sure that the path in the perl script (1st line in the script) matches whatever path was given by the result of your which command. Make sure that the program is executable (use chmod 755 filename) where filename = process_mdout.perl.

```
./process_mdout.perl md1.out md2.out
```

The resulting files are readable by the Grace program (http://plasma-gate.weizmann.ac.il/Grace/) or as space delimited files in Excel.

We will take a look at summary.EPTOT (potential energy plot). Here is our plot for a 500 ps simulation…



The potential energy fluctuates mildly throughout the simulation. The general trend is toward lower energy after a jump in energy during the restrained dynamics (water pre-equilibration), which is a good sign that the dynamics is leading toward lower energy conformation(s). Plot other files. Use Microsoft Excel and read in the file as a space delimited file.

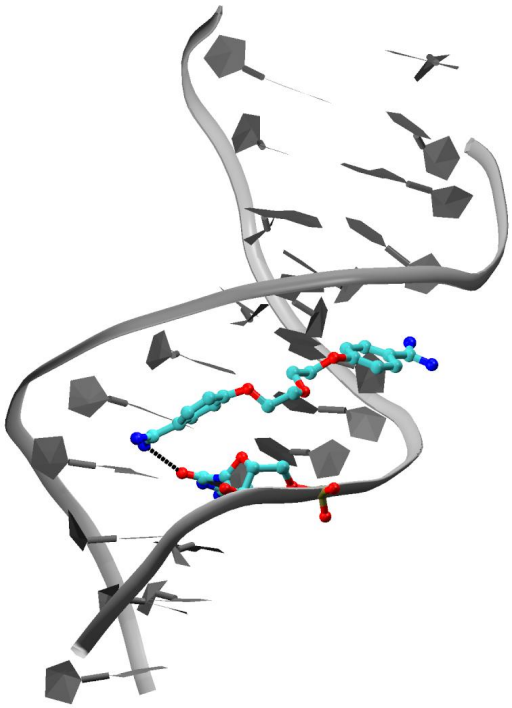
summary.TEMP gives the temperature fluctuation with time.
summary.PRES gives the pressure fluctuation with time.

Analysis of hydrogen bonds over the course of the trajectory. Use a molecular viewer (like VMD) to examine your structure for hydrogen bonds between the drug molecule and the DNA. Pick out a key hydrogen bond of interest to analyze. Use the following input file as a template to ptraj. (CAUTION: Your atom names for your drug molecule (PET) might be different than what is listed in the template below!)

```
trajin pet_md2.mdcrd
donor DT O2
acceptor PET N1 H20
hbond distance 3.5 angle 120 donor DT O2 acceptor PET N1 H20 neighbor 2 series hbond
```

DONOR/ACCEPTOR – use to specify the donor acceptor heavy atoms.
DISTANCE – use to specify the cutoff distance in angstroms between the heavy atoms participating in the interaction.
ANGLE – The H-bond angle cutoff (donor-H-acceptor) in degrees.
SERIES – Directs H-bond data summary to STDOUT.

```
ptraj pet.top < hbond.in > pet_hbond.dat
```



View detailing the hydrogen bond being analyzed. (Image created using VMD [11] and Raster3D [12]) Output from our hbond analysis …

```
          DONOR          ACCEPTORH       ACCEPTOR
  atom# :res@atom    atom# :res@atom atom# :res@atom %occupied  distance       angle
|   246    :8@O2   |   803    :25@H20   801    :25@N1   |  52.80  2.956 ( 0.16)  24.90 (13.42)
```

The statistical analysis in the pet_hbond.dat file will be most important. Look for those H-bonds with high % occupancy (> 50%). These are the more stable H-bonds. The higher the % occupancy; the better. When analyzing H-bond data, it is best to establish reasonable guidelines for the distance and angle cutoffs. A recent paper by Chapman et al. provides a nice discussion of hydrogen bond criteria. [13]

The AMBPDB Conversion Program

How to use the **ambpdb** program to convert an amber restart coordinate file to a PDB file. For example:

```
ambpdb -aatm -bres -p pet_vac.top < pet_avmin.rst > pet_avmin.pdb
```

The **–aatm** flag insures that the hydrogen atom names conform to PDB standard and the **–bres** flag insures that the residue names conform to PDB standard.

The RMS plot. We will use the ptraj program.

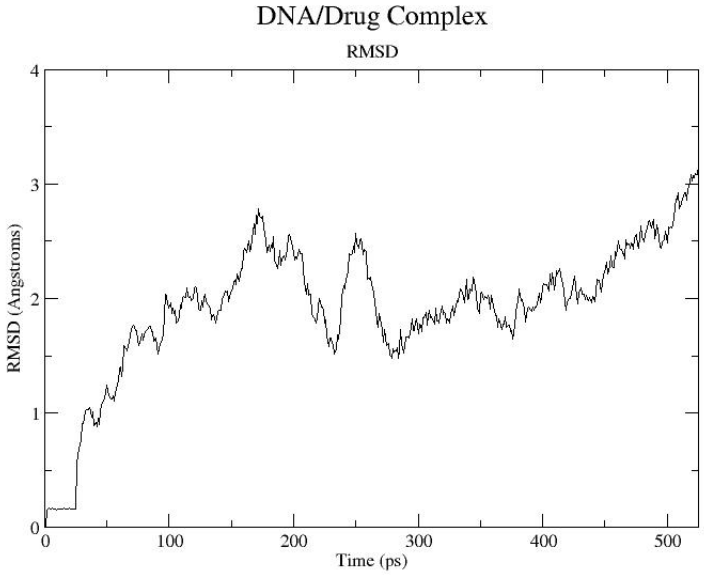
Contents of rms.in

```
trajin pet_md1.mdcrd
trajin pet_md2.mdcrd
rms first out pet_rms.dat :1-24 time 1.0
```

trajin – specifies trajectory file to process
rms – computed RMS fit to the first structure of the first trajectory read in.
out – specifies name of output file
:1-24 – perform rmsd analysis on residues 1 through 24 only (DNA only).

ptraj pet.top < rms.in

xmgrace pet_rms.dat



The model has not equilibrated after 500 ps. There is a region of some stability between 300 and 450 ps.

avg.in

```
trajin pet_md2.mdcrd 300 450
center: 1-25
image center familiar
rms first mass out av_rms.dat :1-25
average pet_avg.rst rest
```

ptraj pet.top < avg.in

Convert the restart file to a pdb file.

ambpdb -aatm -p pet.top < pet_avg.rst.1 > pet_avg.pdb

Remove water and ions with any text editor.

pam_vac.scr

```
source leaprc.ff99
source leaprc.gaff
loadAmberParams pam.frcmod
loadAmberPrep pam_bcc.prep
nap = loadPdb pet_avg.pdb
check nap
saveAmberParm nap pet_avg.top pet_avg.crd
Quit
```

```
tleap -s -f pam_vac.scr
```

Minimize in-vacuo (min_vac.in)

```
oxytocin: in vacuo minimisation prior to MD
 &cntrl
  imin   = 1,
  maxcyc = 2500,
  ncyc   = 500,
  ntb    = 0,
  igb    = 0,
  cut    = 12
 /
```

```
nohup sander -O -i min_vac.in -o min_vac.out -p pet_avg.top -c pet_avg.crd -r
pet_avmin.rst &
```

Using X3DNA to examine the DNA structure. (NOTE: Perform this step in a separate directory!)

The X3DNA program is a very useful tool for analyzing the stereochemical and other 3-dimensional aspects of nucleic acids. X3DNA was developed by Xiang-Jun Lu in Wilma Olson's research group at Rutgers University (see http://rutchem.rutgers.edu/~xiangjun/3DNA/ for more information and a copy of the user manual). [14] You must start from a PDB file. Analyze either your average structure or the last frame saved by Amber (i.e. the restrt file converted to a pdb with ambpdb).

```
ambpdb -bres -p file.top < file_md.rst > file_md.pdb
```

First, edit out the drug and all other HETAM records in a text editor. Next, you must add the chain ID. To do this split the DNA chains into two separate files (dna_a.pdb and dna_b.pdb). Use the change-pdb-chain-id.perl script from 3D-Dock as follows…

```
change-pdb-chain-id.perl -pdb dna_a.pdb -old ' ' -new A

change-pdb-chain-id.perl -pdb dna_b.pdb -old ' ' -new B
```

Combine the two chains into one file (e.g. myfile.pdb or dna.pdb) and start with the **find_pair** utility (used to establish the base pair information).

```
find_pair myfile.pdb myfile.inp
```

Use the **analyze** program to carry out the analysis.

```
analyze myfile.inp
```

You now have an analysis of the structure of the DNA from the drug-DNA complex. Look for the data in the *.out file. Perform the same analysis in a separate directory for the crystal structure (166D.pdb). Compare differences in the minor and major groove widths especially in the proximity of where the drug binds (take the average value for basepairs 4-8). Our averages reveal no difference between the model and the x-ray structure.

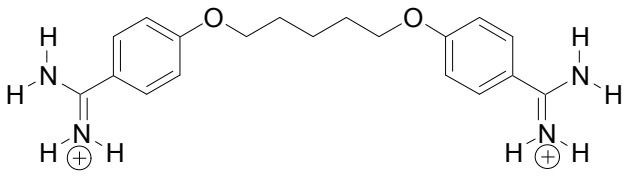
10.7 Å (model P-P distance); vs 10.9 Å (x-ray structure P-P distance)

Questions to consider:

1. The authors (Nunn, et al.; ref #2) claim that the only interaction(s) they observe in their structure is between the amidinium NH and the O4′ of the deoxyribose sugar. What hydrogen bond interactions do you observe from your dynamics run?

2. The authors note that a chain of water molecules exist along the mouth of the minor groove just outside the bound drug. Do you observe a similar pattern in the water structure of your model? (*Hint*: Use the pet_md.restrt file; convert to a PDB using the ambpdb command to view with any viewer – or – use moil-view to view the structure.).

3. Pentamidine has a higher binding affinity for this DNA duplex than does γ-oxa-pentamidine. Make the appropriate structural change and develop a new prep file for pentamidine using antechamber. Perform a dynamics run of the pentamidine complex and the γ-oxa-pentamidine, each for 200 ps duration (i.e. nstlim = 100000). Perform the usual analysis. How does pentamidine compare with γ-oxa-pentamidine in terms of number of hydrogen bonds with the DNA? How do the interaction energies ($E_{int} = E_{vdw} + E_{elec}$) of the two complexes compare (Compare the energies of the average in-vacuo structures only.)? How does the minor groove spacing compare between the two complexes (use 3DNA or measure the distance between P atoms of the phosphates by picking 4 that 'bracket' the drug binding region.)?



Pentamidine

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